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The Influence of Environmental Stress on Cell-Mediated Immune Function

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SUMMARY

An experimental protocol of repeated skin testing with several challenge antigens was utilized to assess the status of cell-mediated immunity (CMI) in all four Phase III crewmembers before, during, and after a 91-day duration stay in the chamber. An identical protocol was used on the same days in four age- and sex-matched control subjects who were not isolated within the chamber. By chamber day 45, all chamber subjects showed either an attenuated response or no response to all of the skin test antigens as determined by a decrease (hypoergy) or absence (anergy) in the CMI score. By chamber day 90, all four chamber subjects had an anergic response to all seven challenge antigens. Control subjects' responses changed variably from baseline, as expected, throughout the entire test period, but the average CMI score did not change significantly. Statistical analyses revealed a significant reduction (48.7% ± 10.1 SEM) in the CMI score in chamber subjects compared to control subjects. The CMI score of chamber crewmembers at 30 days following the period of chamber isolation was slightly reduced (13.1% ± 13.05 SEM), but the reduction was not statistically significant compared to control values. These results indicate that human subjects may suffer a decrease in cell-mediated immune responsiveness when challenged by moderate (91 days) duration isolation within an enclosed chamber. Additionally, the results support the utility of such chamber studies as a test bed for long-duration space missions including lunar/Mars exploration-class and Earth-orbiting space station missions and may further serve as an experimental model for determining the mechanisms underlying the attenuation of CMI function in extended-duration isolation.

INTRODUCTION

Objectives of Experiment

This investigation had two specific aims: (1) to determine if isolation of human subjects within the closed chamber would adversely affect function of the cell-mediated arm of the immune system as assessed by a delayed-type hypersensitivity (DTH) skin response to purposely introduced foreign antigens, and (2) to determine if the closed-chamber test bed is an appropriate ground-based analogue to further investigate the potential effects of isolation on underlying mechanisms that may alter cell-mediated immune function during long-duration space flight or extended stays on an Earth-orbiting space station facility.

Background

The human immune system is composed of multiple interacting elements including contributions from both the humoral and cell-mediated arms. These elements play unique roles and interact in various ways with each other in maintaining the optimum immune status and health of humans. CMI involving sensitized T-lymphocytes is important in defense against certain infectious agents (e.g., viruses and fungi), in surveillance against neoplastic cells, and in regulation of immune function. CMI function testing has traditionally been done by skin testing with cutaneous placement of recall antigens (delayed cutaneous hypersensitivity). By introducing an antigen to which an individual has been previously exposed, the capacity of T-lymphocytes to respond to an antigen in memory can be assessed.

Measurement of cutaneous DTH responses to a battery of commonly encountered antigens is a generally accepted and preferred means of assessing CMI function. In the past, such DTH testing suffered from lack of standardization of testing techniques, number and characterization of reactions, doses employed, and interpretation of reactions and results. A commercially available system (Multitest® CMI device; Pasteur Mériéux Serums et Vaccins, SA, Lyon, France) has solved these problems by providing simultaneous and reproducible application of seven standardized recall antigens as a means of measuring DTH in assessment of CMI. Because of its properties, widespread clinical acceptance, ease of use, and availability of scientific studies from other investigators (2, 3, 4), this system was adopted for this study.

In this investigation, repeated skin testing was utilized in order to determine the functional state of the chamber crew's CMI system over time and compare it to a control group of subjects not exposed to the environmental stress of isolation within the closed chamber.

This process of skin testing and evaluation of cell-mediated immune function has been used in other extreme environments such as Antarctic expeditions (6, 9),

tours of duty in submarines, and during both short- (7, 8) and long-duration (5) space flights. All of these studies have shown that stress can have a negative impact on CMI function. The exact mechanisms underlying these changes are not yet fully understood.

Methods and Materials

Human Subjects

There were two subject groups in this study. The experimental (chamber) group consisted of the four chamber occupants and the control group consisted of four sex- and age-matched volunteers. The test protocol, layman's summary, and informed consent documents were approved by the NASA Johnson Space Center Institutional Review Board prior to commencement of the study. All human subjects (chamber and control) received an informed consent briefing detailing the experimental protocol and risks and signed the informed consent documents before the start of the study. All individuals completed a training session on the proper application of the skin test device and measurement of the results.

CMI Device Description and Procedure

Multitest® CMI (Pasteur Mériéux Serums et Vaccins, SA, Lyon, France) is a disposable applicator made of acrylic resin. It has eight heads with nine tines on each head, linked by a support and loaded with seven different antigens (Tetanus Toxoid, Diptheria Toxoid, Streptococcus Group C, Tuberculin (Old), Candida, Trichophyton, Proteus) and a glycerin control with one antigen or the control per head. The following procedure was used for application of the testing device at each time point:

1) The volar surface of a forearm is cleansed with an alcohol pad and allowed to dry
2) The test device is then placed against the forearm and firmly pressed into the skin. The prongs at the tip of each arm enter the skin and deliver the antigen
3) A rocking motion is used to ensure adequate delivery of the antigens and control
4) The test device is removed, and the area is allowed to dry for 5 minutes
5) A permanent marker is used to outline the skin area tested to allow later observation of the proper sites
6) After 48 hours, each antigen site is evaluated for induration and calipers are used to measure the diameter of the induration along the vertical and horizontal axes
7) The number of antigens that reach at least 2 mm in diameter are considered positive. The sum total millimeters of induration and the number of positive antigens are recorded and used to determine a "CMI score" according to the formula: CMI Score = Sum of Mean Indurations ÷ Number of Positive Antigens.

Test Protocol

At 30 days prior to chamber entry, all subjects had seven specific antigens and one control placed subcutaneously on the volar surface of a forearm utilizing the Multitest® CMI device according to the manufacturer's instructions. Forty-eight hours after antigen placement, the number of positive responses to the seven antigens and to the negative control was observed, the level of induration for each positive antigen was measured by a physican evaluator (Dr. D'Aunno), and the results were recorded.

On day 45 of the chamber stay, the Multitest® CMI device was used to apply the antigens in all subjects. Forty-eight hours later, skin responses in the control group were measured by the physician evaluator. The chamber crew used the Telemedicine Instrumentation Pack to transmit the images of the skin responses to the physician evaluator who coached the chamber crew on measurement of the indurations.

Forty-eight hours prior to the end of the chamber stay, all subjects had a repeat placement of the antigens with the Multitest® CMI device. Upon completion of the 91-day test in the chamber, skin responses were measured by the physician evaluator in both the chamber crewmembers and in the control group.

One month after the chamber study, all subjects had a repeat placement of antigens with the Multitest® CMI device. The results were interpreted 48 hours later by the physician evaluator.

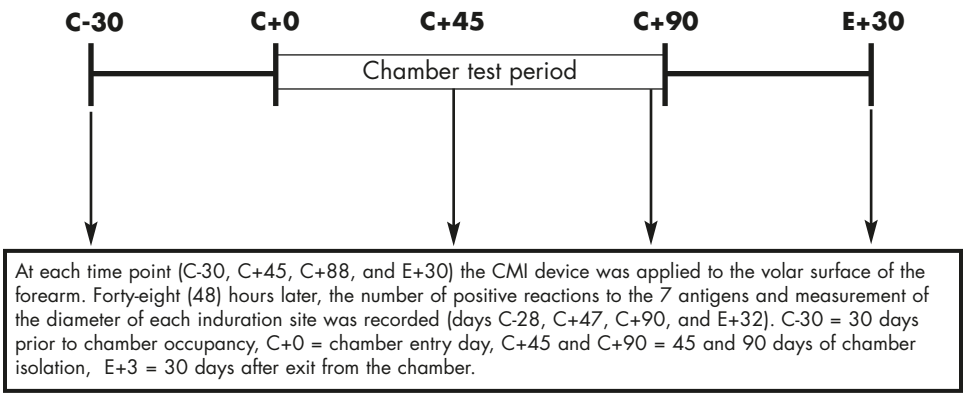


Figure 5.4-1 *Assessment of cell-mediated immunity protocol timeline*

Table 5.4-1 *Experimental test protocol*

Day	C-30	C-28	C+45	C+47	C+88	C+90	E+30	E+32
Action	Antigens Placed	Skin Results Evaluated	Antigens Placed	Skin Results Evaluated	Antigens Placed	Skin Results Evaluated	Antigens Placed	Skin Results Evaluated
Subjects	4 Chamber Crew 4 Control Subjects	4 Chamber Crew 4 Control Subjects	4 Chamber Crew 4 Control Subjects	4 Chamber Crew 4 Control Subjects	4 Chamber Crew 4 Control Subjects	4 Chamber Crew 4 Control Subjects	4 Chamber Crew 4 Control Subjects	4 Chamber Crew 4 Control Subjects

RESULTS

Tables 5.4-2 and 5.4-3 summarize the results in each of the chamber and control subjects at each study time point. The number of antigens that produced a measurable induration, the sum of mean induration measurements for each time point, and the calculated CMI score are given in tabular form for each subject for both the chamber and control groups.

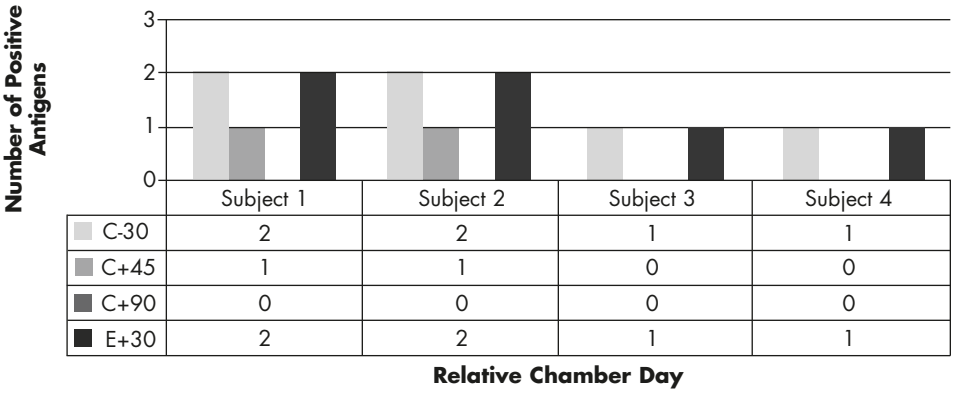
Table 5.4-2 *CMI measurements in control and chamber subjects at C-30 and C-45 days*

	C-30			C+45		
Chamber Group	# of + Antigens	Sum of Mean Indurations	Calculated CMI Score	# of + Antigens	Sum of Mean Indurations	Calculated CMI Score
Subject #						
1	2	6	3	1	2	2
2	2	8	4	1	2	2
3	1	2.3	2.3	0	0	0
4	1	5.5	5.5	0	0	0
Control Group						
Subject #						
5	3	11.7	3.9	3	9.2	3.1
6	4	14.4	3.6	4	14.8	3.7
7	2	12	6	2	12	6.0
8	4	10.8	2.7	6	21.3	3.6

Table 5.4-3 *CMI measurements in control and chamber subjects at C+90 and E+30 days*

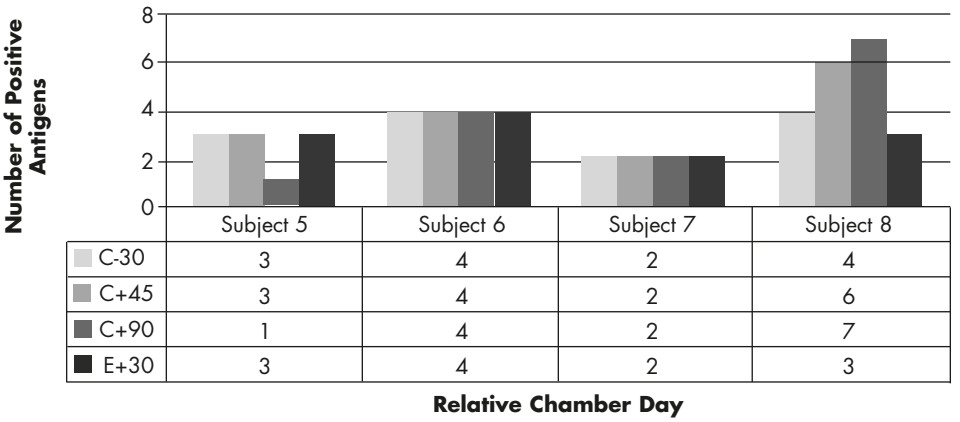
	C+90			E+30		
Chamber Group	# of + Antigens	Sum of Mean Indurations	Calculated Score	# of + Antigens	Sum of Mean Indurations	Calculated CMI Score
Subject #						
1	0	0	0	2	6	3
2	0	0	0	2	7.5	3.75
3	0	0	0	1	2.4	2.4
4	0	0	0	1	4.1	4.1
Control Group						
Subject #						
5	1	4.1	4.1	3	10.6	3.5
6	4	14.1	3.5	4	15	3.8
7	2	11.9	6.0	2	12.2	6.1
8	7	23.5	3.4	3	10.6	3.5

The number of positive reactions to the seven antigens is shown for each of the chamber (Figure 5.4-2) and control (Figure 5.4-3) subjects at each of the study time points. The chamber subjects had fewer responses to the seven antigens at the C-30 time point compared to the control subjects. By chamber day 45 (C+45), the chamber subjects showed hypoergic responses, and by chamber day 90 (C+90) all chambers subjects exhibited anergy to the seven challenge antigens. The chamber subjects had returned nearly to their prechamber baselines within 30 days of exiting the chamber. The control group subjects responded to more antigens at the C-30 time point and showed variable responses but only minor changes throughout the study period.



	Subject 1	Subject 2	Subject 3	Subject 4
C-30	2	2	1	1
C+45	1	1	0	0
C+90	0	0	0	0
E+30	2	2	1	1

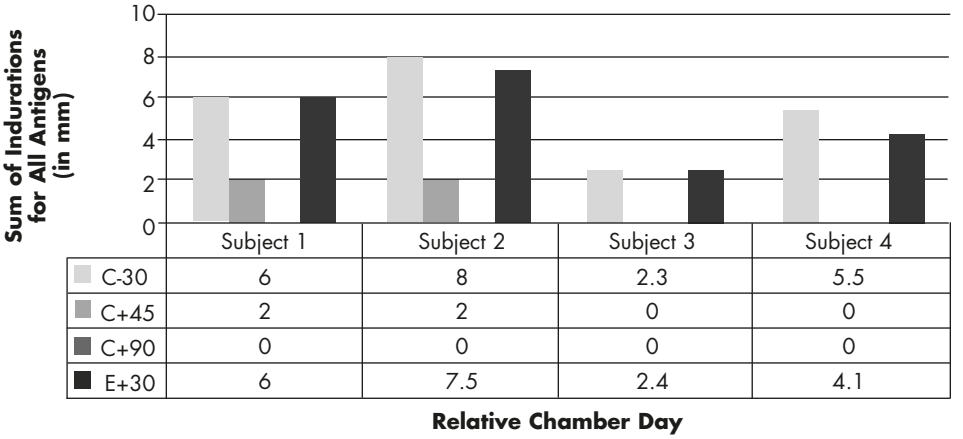
Figure 5.4-2 *Number of positive reactions to seven antigens in four chamber subjects by relative chamber day*



	Subject 5	Subject 6	Subject 7	Subject 8
C-30	3	4	2	4
C+45	3	4	2	6
C+90	1	4	2	7
E+30	3	4	2	3

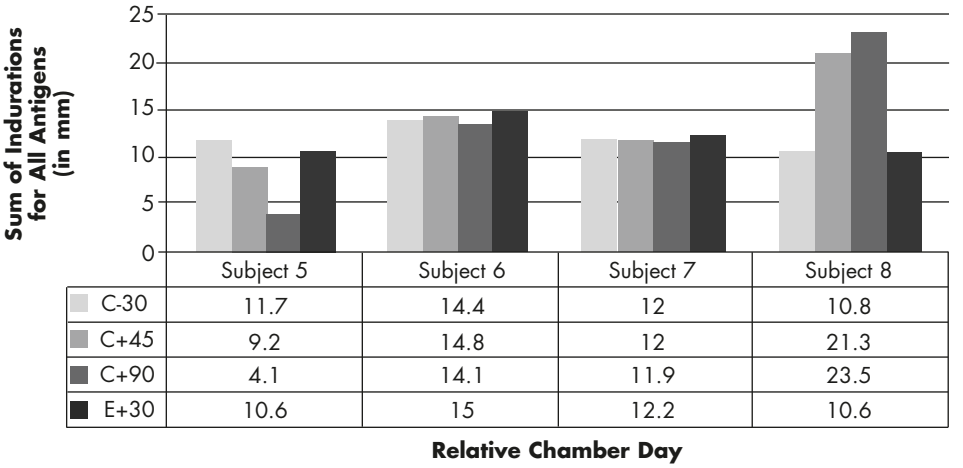
Figure 5.4-3 *Number of positive reactions to seven antigens in four control subjects by relative chamber day*

The sum of indurations of positive responses to the seven challenge antigens is shown for the chamber (Figure 5.4-4) and control (Figure 5.4-5) subjects. Since the chamber subjects responded initially (C-30) to fewer antigens, the baseline value for the sum of the indurations in the chamber subjects is less than that for the control group. The chamber subjects showed a loss of reactivity to most of the antigens by chamber day C+45 and had no response to any of the antigens and thus no measurable indurations at chamber day C+90. By 30 days after exit from the chamber (E+30), the measured sum of indurations had returned to near baseline level in the chamber subjects. The control subjects showed a variable response throughout the study. One control subject exhibited a slightly attenuated response on chamber day C+45 while another showed increased responses at chamber days C+45 and C+90. On day E+30, all control subjects had values similar to pre-chamber baseline measurements.



	Subject 1	Subject 2	Subject 3	Subject 4
C-30	6	8	2.3	5.5
C+45	2	2	0	0
C+90	0	0	0	0
E+30	6	7.5	2.4	4.1

Figure 5.4-4 *Sum of indurations (in mm) from all positive skin reactions to seven antigens in four chamber subjects by relative chamber day*



	Subject 5	Subject 6	Subject 7	Subject 8
C-30	11.7	14.4	12	10.8
C+45	9.2	14.8	12	21.3
C+90	4.1	14.1	11.9	23.5
E+30	10.6	15	12.2	10.6

Figure 5.4-5 *Sum of indurations (in mm) from all positive skin reactions to seven antigens in four control subjects by relative chamber day*

The most interesting and meaningful results (Average CMI score) for both the control and chamber groups are summarized in Figure 5.4-6. The average CMI score for the control subjects varied little throughout the entire study period (range 4.05 to 4.23). However, the chamber subjects as a group showed a profound decrement in their average CMI scores on chamber days C+45 and C+90 with no response to any of the seven challenge antigens noted in any of the four chamber subjects on chamber day C+90. The average CMI score of the chamber group had returned to near the baseline level at 30 days postchamber (E+30).

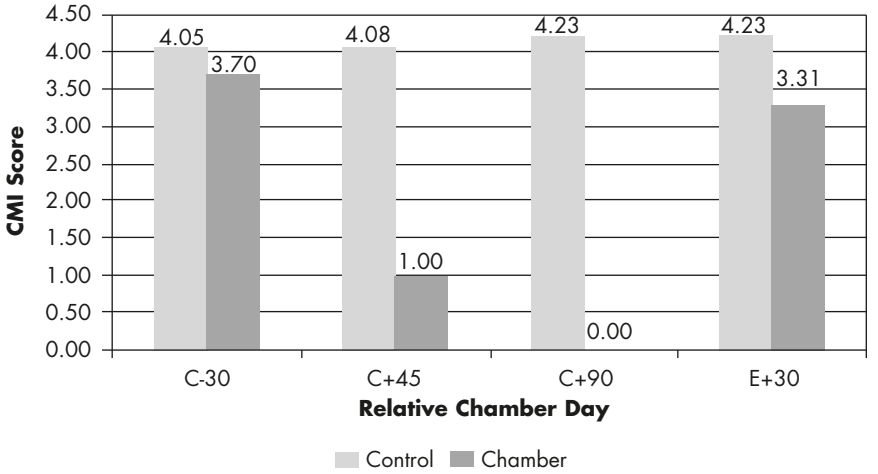


Figure 5.4-6 *Mean CMI scores in 4 chamber and 4 control subjects by relative chamber day. The mean CMI score for control subjects was relatively unchanged throughout the study period*

The control and experimental groups in this study were small (n = 4 for each group), and since the CMI score was a calculated value (CMI Score = sum of diameters of induration/number of positive reactions to antigens) based on whether a skin reaction occurred resulting in an area of induration, then the case in which there were no skin reactions to any of the seven antigens was problematic in that the mathematical calculation was not defined due to division by zero. For calculation of a CMI score when there was no response to any of the antigens, the CMI score was recorded as 0. These factors required a thoughtful approach in order to provide useful statistical comparisons. An expert statistician was consulted for guidance, and the approach taken was to regard the study as having a single perturbation—isolation within the chamber. A statistical model was developed in which data were combined from both the control and chamber groups for the subjects not confined to the chamber (n = 20; all measurements for the control group at all study time points plus the prechamber measurements from the chamber group; these data comprised the control data set). The data obtained on both in-chamber study time points (C + 45 and C + 90) were combined for the chamber group to comprise a data set of values (n = 8) measured during chamber isolation; the in-chamber data set). Finally, a third data set consisted of the data from measurements made postchamber on the chamber group 30 days after exit from the chamber (E + 30; n = 4; the postchamber data set). The three data sets were analyzed for variance and the variance expressed as a percent change ± SEM from the control data set calculated from measurements made in subjects not isolated in the chamber. Figure 5.4-7 shows the results of these statistical comparisons. The chamber subjects had a nearly 49% decrease in their CMI scores during the chamber

stay which was statistically different from the control data set at p = 0.002. The CMI scores of the chamber subjects were still decreased from control values by approximately 13% at time point E+30 (30 days after exiting the chamber), but the difference was not significant.

DISCUSSION

The effects of stress on the human immune system have been studied in numerous environments including Antarctic expeditions (6, 9) and in spacecraft during both short- (7, 8) and long-duration (5) Earth-orbital missions. These studies have collectively shown decrements in human immune function associated with these environments including decreased cell-mediated immune function.

The primary aim of the current study was to determine if isolation of human subjects within a closed chamber over a period of 91 days would adversely affect function of the cell-mediated arm of the immune system as assessed by delayed-type hypersensitivity (DTH) skin responses to specific test antigens utilizing a commercially available and scientifically validated cutaneous test system (2, 3, 4). The CMI scores were significantly decreased (-48.7% ± 10.1 SEM; p= 0.002; n = 8) for the chamber subjects during chamber isolation. The CMI scores were decreased below the control level (-13.1% ± 13.05 SEM) for the isolated chamber subjects at 30 days after exit from the chamber but were not statistically different from the control values at this time point.

Based on previous studies (5, 6, 7, 8, 9) in analogue environments, the decrement in CMI function during chamber isolation was not unexpected. An interesting aspect of this particular study was that the subjects also participated in an exercise study (see Chapter 5.2: Exercise Countermeasures Demonstration Projects During the Lunar-Mars Life Support Test Project Phases IIa and III). For Phase III, the chamber subjects completed a battery of exercise countermeasures including both aerobic and resistive exercises each day for six days, resting on the seventh day. For aerobic exercise, a cycle protocol was performed three days per week and a steady-state treadmill protocol was added on the remaining three exercise days. Additionally, an upper- and lower-body resistance exercise protocol was performed. The benefits of exercise on the immune system are well documented, and thus it would be predicted that the negative effects of chamber isolation should be at least partially offset by participation in daily exercise. However, the type, level, and duration of exercise seems to be important in achieving increased immune responsiveness, and excessive levels of certain types of exercise may contribute to decrements in immune function (1).

The short-term and long-term effects of decreased CMI in isolated human subjects are not known. Since CMI plays important roles in combating infectious agents (e.g., viruses and fungi), in surveillance against neoplastic cells, and in regulation of immune function, possible consequences include increased susceptibility to acute and chronic infections, increased cancer risk, and immune dysregulation. The level of

these increased risks associated with decreased CMI function in conjunction with isolation and other still poorly understood environmental, physiological, and psychological factors is not known. Additional prospective and retrospective longitudinal studies are required to better understand underlying mechanisms and the level of risks associated with decreased CMI function in persons living in isolated environments. The role of exercise and the specific types, intensity levels, and duration in modulating the immune response during isolation requires further investigation.

The final aim of the project was to determine if the closed-chamber test bed is an appropriate ground-based analogue to further investigate the potential effects of isolation on underlying mechanisms that may alter cell-mediated immune function during long-duration space flight or extended stays at an Earth-orbiting space station facility. Experience and knowledge gained by this study supports the use of closed chamber studies for this purpose.

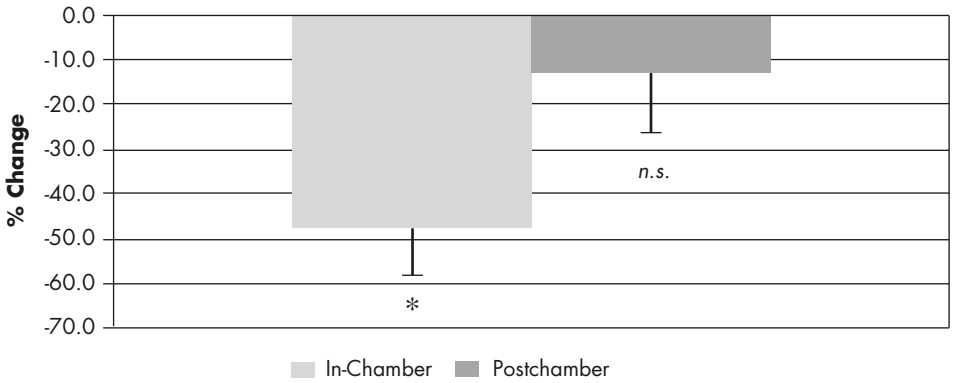


Figure 5.4-7 *Change (in percent ± SEM) from control (n=20) in composite CMI score for in-chamber measurements (n=8) and postchamber measurements (n=4). *Significant difference (p=0.002). n.s. = not significant*

REFERENCES

1. Bruunsgaard H, Harkopp A, Mohr T, Konradsen H, Heron I, Mordhorst CH, Pedersen BK. 1997. In vivo cell-mediated immunity and vaccination response following prolonged, intense exercise. *Med Sci Sports Exerc* 29:1176-1181.
2. Frazer IH, Collins EJ, Fos JS, Jones B, Oliphant RC, MacKay IR. 1985. Assessment of delayed-type hypersensitivity in man: a comparison of the "Multitest" and conventional intradermal injection of six antigens. *Clin Immunol Immunopath* 35:182-190.
3. Kniker WT. Amderson CT, Roumiantzeff M. 1979. The multi-test system: a standardized approach to evaluation of delayed hypersensitivity and cell mediated immunity. *Ann Allergy* 43:73-79.
4. Kniker WT, Anderson CT, McBryde JL, Roumiantzeff M, Lesourd B. 1984. Multitest CMI for standardized measurement of delayed cutaneous hypersensitivity and cell-mediated immunity. Normal values and propsed scoring system for healthy adults in the USA. *Ann Allergy* 52:75-82.
5. Konstantinova IV, Rykova MP, Lesnyak AT, Antropova EA. 1993. Immune changes during long-duration missions. *J Leukocyte Biol* 54:189-201.
6. Metha SK, Pierson DL, Cooley H, Dubow R, Lugg D. 2000. Epstein-Barr virus reactivation associated with diminished cell-mediated immunity in Antarctic expeditioners. *J Med Virol* 61:235-240.
7. Taylor GR, Janney RP. 1992. In vivo testing confirms a blunting of the human cell-mediated immune system during space flight. *Adv Space Bio Med* 6:1-32.
8. Taylor GR. 1993. Immune changes during short-duration missions. *J Leukocyte Biol* 54:202-208.
9. Williams KL, Climie A, Muller HK, Lugg DJ. 1986. Cell-mediated immunity in healthy adults in Antarctica and the sub Antarctic. *J Clin Lab Immunol* 20:43-49.